# WEST Search History

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DATE: Monday, July 26, 2004

| Hide? | Set Name | Query | Hit Count |
|---|---|---|---|
| | | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ* | |
| ☐ | L1 | 10/069236 | 0 |
| | | *DB=PGPB; PLUR=YES; OP=ADJ* | |
| ☐ | L2 | 10/069236 | 0 |
| | | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ* | |
| ☐ | L3 | 5436143.pn. | 2 |
| ☐ | L4 | primer extension same template dependent | 334 |
| ☐ | L5 | L4 same nascent | 1 |
| ☐ | L6 | nascent near (oligonucleotide or primer or polynucleotide) | 150 |
| ☐ | L7 | L6 same ((template dependent) near (manner or orientation or direction)) | 5 |
| ☐ | L8 | kless-H$.in. | 7 |
| ☐ | L9 | L8 | 7 |
| ☐ | L10 | L6 and ((template dependent) near (manner or orientation or direction)) | 7 |
| ☐ | L11 | L6 and (template dependent) | 14 |
| ☐ | L12 | ((template dependent) near (manner or orientation or direction)) | 569 |
| ☐ | L13 | L12 and primer extension | 515 |
| ☐ | L14 | L13 and ((oligonucleotide or primer or polynucleotide) near hybridiz$ near template) | 19 |
| ☐ | L15 | L13 and template dependent polymerase | 30 |
| ☐ | L16 | L15 and oligonucleotide triphosphate | 5 |
| ☐ | L17 | 4683195.pn. or 4683202.pn. or 4965188.pn. or 5075216.pn. or 4795699.pn. or 5888813.pn. | 10 |
| ☐ | L18 | l13 and monomer | 398 |
| ☐ | L19 | L18 and (4N dNTP) | 0 |
| ☐ | L20 | L18 and (dNTPs) | 268 |
| ☐ | L21 | L20 and integer | 6 |
| ☐ | L22 | L20 and oligonucleotide triphosphate | 4 |
| ☐ | L23 | L18 and oligonucleotide triphosphate | 5 |
| ☐ | L24 | L13 and oligonucleotide triphosphate | 5 |
| ☐ | L25 | oligonucleotide triphosphate | 13 |
| ☐ | L26 | 10/069236 | 0 |
| | | *DB=PGPB; PLUR=YES; OP=ADJ* | |

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| ☐ | L27 | 10/069236 | 0 |
| | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ* | | |
| ☐ | L28 | 5436143.pn. | 2 |
| ☐ | L29 | primer extension same template dependent | 334 |
| ☐ | L30 | L29 same nascent | 1 |
| ☐ | L31 | nascent near (oligonucleotide or primer or polynucleotide) | 150 |
| ☐ | L32 | L31 same ((template dependent) near (manner or orientation or direction)) | 5 |
| ☐ | L33 | kless-H$.in. | 7 |
| ☐ | L34 | L33 | 7 |
| ☐ | L35 | L31 and ((template dependent) near (manner or orientation or direction)) | 7 |
| ☐ | L36 | L31 and (template dependent) | 14 |
| ☐ | L37 | ((template dependent) near (manner or orientation or direction)) | 569 |
| ☐ | L38 | L37 and primer extension | 515 |
| ☐ | L39 | L38 and ((oligonucleotide or primer or polynucleotide) near hybridiz$ near template) | 19 |
| ☐ | L40 | L38 and template dependent polymerase | 30 |
| ☐ | L41 | L40 and oligonucleotide triphosphate | 5 |
| ☐ | L42 | 4683195.pn. or 4683202.pn. or 4965188.pn. or 5075216.pn. or 4795699.pn. or 5888813.pn. | 10 |
| ☐ | L43 | L38 and monomer | 398 |
| ☐ | L44 | L43 and (4N dNTP) | 0 |
| ☐ | L45 | L43 and (dNTPs) | 268 |
| ☐ | L46 | L45 and integer | 6 |
| ☐ | L47 | L45 and oligonucleotide triphosphate | 4 |
| ☐ | L48 | L43 and oligonucleotide triphosphate | 5 |
| ☐ | L49 | L38 and oligonucleotide triphosphate | 5 |
| ☐ | L50 | oligonucleotide triphosphate | 13 |
| ☐ | L51 | dinucleotide triphosphate | 23 |
| ☐ | L52 | L51 and primer extension | 2 |
| ☐ | L53 | thmidylyl near dinucleotide | 0 |
| ☐ | L54 | 5432272.pn. | 2 |
| ☐ | L55 | TpT | 3521 |
| ☐ | L56 | L55 or thymidylyl near$3 thymidine | 3521 |
| ☐ | L57 | L55 and thymidylyl near$3 thymidine | 0 |
| ☐ | L58 | thymidylyl near$3 thymidine | 0 |
| ☐ | L59 | thymidylyl | 75 |
| ☐ | L60 | L59 and TpT3p | 0 |
| ☐ | L61 | tpT and l59 | 5 |

| | | | |
|---|---|---|---|
| ☐ | L62 | l59 and primer extension | 3 |
| ☐ | L63 | deoxycytidylyl or deoxyadenylyl | 27 |
| ☐ | L64 | L63 and primer extension | 1 |

END OF SEARCH HISTORY

L14: Entry 1 of 19 File: PGPB Jul 1, 2004

DOCUMENT-IDENTIFIER: US 20040126789 A1
TITLE: Compositions and methods for synthesizing nucleic acids

Abstract Paragraph:
Also disclosed are methods for using such compositions in nucleic acid synthesis, amplification and sequencing, where various combinations of anti-RT antibodies, anti-DNAP antibodies and/or SSBs can improve the yield and/or homogeneity of primer extension products.

Summary of Invention Paragraph:
[0008] DNA polymerases (DNAPs) synthesize DNA molecules that are complementary to all or a portion of a nucleic acid template (preferably a DNA template). Upon hybridization of a primer to a DNA template to form a primed template, DNA polymerases can add nucleotides to the 3' hydroxy end of the primer in a template dependent manner (i.e., depending upon the sequence of nucleotides in the template). Thus, in the presence of deoxyribonucleoside triphosphates (dNTPs) and a primer, a new DNA molecule, complementary to all or a portion of one or more nucleic acid templates, can be synthesized.

Summary of Invention Paragraph:
[0009] DNAPs have been used to detect nucleic acids in biological and environmental test samples, e.g., using polymerase chain reaction (PCR)-based nucleic acid synthesis (see e.g., U.S. Pat. Nos. 4,683,195; 4,683,202 and 4,965,188). In PCR-based nucleic acid synthesis, one or more templates are hybridized to smaller complementary "primer" nucleic acids in the presence of a thermostable DNAP and deoxyribonucleoside triphosphates. Upon hybridization of a primer and a template to form a "primed template complex," DNAP can extend the primer in a template directed manner to yield a primer extension product. Primer extension products can then serve as templates for nucleic acid synthesis. Upon denaturation, the primer extension products can hybridize with primers to form primed template complexes that can serve as DNAP substrates. Cycles of hybridization, primer extension and denaturation can be repeated many times to exponentially increase the number of primer extension products. Thus, PCR-based nucleic acid synthesis is a very sensitive technique for detecting template nucleic acids.

Summary of Invention Paragraph:
[0010] The yield and homogeneity of primer extension products made by DNAP can be adversely affected by "mispriming" (i.e., hybridization of primers to inappropriate regions of the template, or to non-template nucleic acids). Primers are designed to hybridize to a specific region of a template nucleic acid. Mispriming can occur when nucleic acid synthesis mixtures containing template, primers, DNAP and nucleotides are maintained at lower temperatures (e.g., during manufacture, shipping, or storage). Extension of misprimed nucleic acids can obscure properly primed primer extension products (i.e., produce high background). In addition, diversion of nucleic acid synthesis reaction constituents to extend misprimed nucleic acids can reduce the yield of properly primed primer extension products, reducing the sensitivity of detection.

Summary of Invention Paragraph:
[0011] The invention features compositions and methods for synthesizing nucleic

acids. The methods and materials of the invention can enhance the yield and/or homogeneity of primer extension products made by DNAPs.

Brief Description of Drawings Paragraph:
[0028] FIG. 8: Scan profile of alkaline agarose gel electrophoresis for primer extension products by Taq DNA polymerase using a specific primer and single stranded circular M13mp19 DNA as a template, in the presence or the absence of AccuPrime protein: (A) primer extension in the absence of AccuPrime protein; (B) with 50 ng AccuPrime protein/50 ml rxn; (C) with 100 ng AccuPrime protein/50 ml rxn; and, (D) with 100 ng MthSSB/50 ml rxn. Results show that in the presence of 100 ng of AccuPrime protein in 50 ml rxn, the peak population of extension products shifted toward lower molecular weight indicating the polymerase extending the primer shorter in the presence of AccuPrime protein than those in the control. This phenomenon was most obvious at 1.5 min time point. The second peak showing on top of the gel in the bottom panels (C and D) is the primer from the top panel.

Detail Description Paragraph:
[0067] The invention provides methods and materials for nucleic acid synthesis (e.g., PCR-based nucleic acid synthesis). The invention is based, in part, on the surprising discovery that the yield and/or homogeneity of primer extension products made by DNAP can be enhanced by including combinations of anti-DNAP antibodies and/or single strand DNA binding proteins (preferably thermostable SSBs) in nucleic acid synthesis mixtures. Nucleic acid synthesis mixture constituents, nucleic acid synthesis methods, and kits useful for performing the same are described herein, along with a brief glossary of terms commonly used by those skilled in the art of molecular biology.

Detail Description Paragraph:
[0069] Template. A template is a single stranded nucleic acid that, when a part of a primer-template complex, can serve as a substrate for DNAP or RT. A nucleic acid synthesis mixture can include a single type of template, or can include templates having different nucleotide sequences. By using primers specific for particular templates, primer extension products can be made for a plurality of templates in a nucleic acid synthesis mixture. The plurality of templates can be present within different discrete nucleic acids, or can be present within a discrete nucleic acid.

Detail Description Paragraph:
[0072] Primer. A primer is a single stranded nucleic acid that is shorter than a template, and that is complementary to a segment of a template. A primer can hybridize to a template to form a primer-template complex (i.e., a primed template) such that a DNAP can synthesize a nucleic acid molecule (i.e., primer extension product) that is complementary to all or a portion of a template.

Detail Description Paragraph:
[0073] Primers typically are 12 to 60 nucleotides long (e.g., 18 to 45 nucleotides long), although they may be shorter or longer in length. A primer is designed to be substantially complementary to a cognate template such that it can specifically hybridize to the template to form a primer-template complex that can serve as a substrate for DNAP to make a primer extension product. In some primer-template complexes, the primer and template are exactly complementary such that each nucleotide of a primer is complementary to and interacts with a template nucleotide. Primers can be made as a matter of routine by those skilled in the art (e.g., using an ABI DNA Synthesizer from Applied Biosystems or a Biosearch 8600 or 8800 Series Synthesizer from Milligen-Biosearch, Inc.), or can be obtained from a number of commercial vendors.

Detail Description Paragraph:
[0074] DNA polymerase (DNAP). A DNA polymerase is an enzyme that can add deoxynucleoside monophosphate molecules to the 3' hydroxy end of a primer in a

primer-template complex, and then sequentially to the 3' hydroxy end of a growing primer extension product in a template dependent manner (i.e., depending upon the sequence of nucleotides in the template). DNAPs typically add nucleotides that are complementary to the template being used, but DNAPs may add noncomplementary nucleotides (mismatches) during the polymerization or synthesis process. Thus, the synthesized nucleic acid strand may not be completely complementary to the template. DNAPs may also make nucleic acid molecules that are shorter in length than the template used. DNAPs have two preferred substrates: one is the primer-template complex where the primer terminus has a free 3'-hydroxyl group, the other is a deoxynucleotide 5'-triphosphate (dNTP). A phosphodiester bond is formed by nucleophilic attack of the 3'-OH of the primer terminus on the .alpha.-phosphate group of the dNTP and elimination of the terminal pyrophosphate. DNAPs can be isolated from organisms as a matter of routine by those skilled in the art, and can be obtained from a number of commercial vendors.

Detail Description Paragraph:
[0081] Primer extension product. A primer extension product is a nucleic acid that includes a primer to which DNAP has added one or more nucleotides. Primer extension products can be as long as, or shorter than the template of a primer-template complex.

Detail Description Paragraph:
[0082] Amplifying. Amplifying refers to an in vitro method for increasing the number of copies of a nucleic acid with the use of a DNAP. Nucleic acid amplification results in the addition of nucleotides to a primer or growing primer extension product to form a new molecule complementary to a template. In nucleic acid amplification, a primer extension product and its template can be denatured and used as templates to synthesize additional nucleic acid molecules. An amplification reaction can consist of many rounds of replication (e.g., one PCR may consist of 5 to 100 "cycles" of denaturation and primer extension). General methods for amplifying nucleic acids are well-known to those of skill in the art (see e.g., U.S. Pat. Nos. 4,683,195; 4,683,202; and 4,800,159; Innis, M. A., et al., eds., PCR Protocols: A Guide to Methods and Applications, San Diego, Calif.: Academic Press, Inc. (1990); Griffin, H., and A. Griffin, eds., PCR Technology: Current Innovations, Boca Raton, Fla.: CRC Press (1994)). Amplification methods that can be used in accord with the present invention include PCR (U.S. Pat. Nos. 4,683,195 and 4,683,202), Strand Displacement Amplification (SDA; U.S. Pat. No. 5,455,166; EP 0 684 315), Nucleic Acid Sequenced-Based Amplification (NASBA; U.S. Pat. No. 5,409,818; EP 0 329 822).

Detail Description Paragraph:
[0126] Methods for synthesizing nucleic acids. Compositions of the invention can be used to improve the yield and/or homogeneity of primer extension products made by DNAP during nucleic acid synthesis (e.g., during first strand synthesis, cDNA synthesis, amplification and combined cDNA synthesis/amplification reactions).

Detail Description Paragraph:
[0127] Compositions of the invention may be used, e.g., in "hot-start" nucleic acid synthesis, where a reaction is set up at a temperature such that anti-DNAP antibodies and/or anti-RT antibodies can exhibit nucleic acid synthesis and where nucleic acid synthesis subsequently is initiated by increasing the temperature to reduce inhibition by the anti-DNAP antibodies and/or anti-RT antibodies. Thus, the invention provides a method for synthesizing a nucleic acid involving: (a) mixing one or more templates with one or more anti-DNAP antibodies and/or one or more anti-RT antibodies and/or one or more SSBs (or combinations thereof) to form a mixture; (b) incubating the mixture under conditions sufficient to inhibit or prevent nucleic acid synthesis; and (c) incubating the mixture under conditions sufficient to make one or more nucleic acid molecules complementary to all or a portion of said templates (i.e., a primer extension product). Reaction conditions sufficient to allow nucleic acid synthesis (e.g., pH, temperature, ionic strength,

and incubation time) can be optimized according to routine methods known to those skilled in the art and may involve the use of one or more primers, one or more nucleotides, one or more buffers or buffering salts, one or more RTs and/or one or more DNAPs (or combinations thereof).

Detail Description Paragraph:
[0130] In another aspect, the invention provides a method for synthesizing a nucleic acid involving: (a) mixing one or more templates with two or more (three or more, four or more, five or more, six or more, etc.) SSBs to form a mixture; and (b) incubating the mixture under conditions sufficient to make a nucleic acid complementary to all or a portion of the templates (i.e., a primer extension product). Reaction conditions sufficient to allow nucleic acid synthesis (e.g., pH, temperature, ionic strength, and incubation time) can be optimized according to routine methods known to those skilled in the art and may involve the use of one or more primers, one or more nucleotides, one or more buffers or buffering salts, one or more RTs and/or one or more DNAPs (or combinations thereof).

Previous Doc Next Doc Go to Doc#

# Hit List

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**Search Results -** Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 6670461 B1

**Using default format because multiple data bases are involved.**

L64: Entry 1 of 1 File: USPT Dec 30, 2003

US-PAT-NO: 6670461
DOCUMENT-IDENTIFIER: US 6670461 B1

TITLE: Oligonucleotide analogues

DATE-ISSUED: December 30, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Wengel; Jesper | Odense | | | DK |
| Nielsen; Poul | Odense | | | DK |

US-CL-CURRENT: 536/23.1; 536/22.1, 536/24.3, 536/25.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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| Term | Documents |
|---|---|
| PRIMER | 118468 |
| PRIMERS | 72604 |
| EXTENSION | 976015 |
| EXTENSIONS | 249366 |
| (63 AND (PRIMER ADJ EXTENSION)).PGPB,USPT,USOC,EPAB,JPAB,DWPI. | 1 |
| (L63 AND PRIMER EXTENSION ).PGPB,USPT,USOC,EPAB,JPAB,DWPI. | 1 |

**Display Format:** - Change Format

Previous Page Next Page Go to Doc#

HIGH FIDELITY DNA SYNTHESIS BY THE THERMUS-AQUATICUS DNA POLYMERASE.

AUTHOR(S): ECKERT K A [Reprint author]; KUNKEL T A
CORPORATE SOURCE: LAB MOL GENETICS, NATL INST ENVIRONMENTAL HEALTH SCIENCES, PO BOX 12233, RESEARCH TRIANGLE PARK, NC 27709, USA

SOURCE: Nucleic Acids Research, (1990) Vol. 18, No. 13, pp. 3739-3744.
CODEN: NARHAD. ISSN: 0305-1048.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 22 Sep 1990
Last Updated on STN: 23 Sep 1990

AB We demonstrate that despite lacking a 3'→5' proofreading exonuclease, the Thermus aquaticus (Taq) DNA polymerase can catalyze highly accurate DNA synthesis in vitro. Under defined reaction conditions, the error rate per nucleotide polymerized at 70° C can be as low as 10-5 for base substitution errors and 10-6 for frameshift errors. The frequency of mutations produced during a single round of DNA synthesis of the lac Zα gene by Taq polymerase responds to changes in dNTP concentration, pH, and the concentration of MgCl2 relative to the total concentration of deoxynucleotide triphosphates present in the reaction. Both base substitution and frameshift error rates of <1/100,000 were observed at pH 5-6 (70° C) or when MgCl2 and deoxynucleotide triphosphates were present at equimolar concentrations. These high fidelity reaction conditions for DNA synthesis by the Taq polymerase may be useful for specialized uses of DNA amplified by the polymerase chain reaction.

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 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  May 12   EXTEND option available in structure searching
 NEWS  4  May 12   Polymer links for the POLYLINK command completed in REGISTRY
 NEWS  5  May 27   New UPM (Update Code Maximum) field for more efficient patent
                   SDIs in CAplus
 NEWS  6  May 27   CAplus super roles and document types searchable in REGISTRY
 NEWS  7  Jun 22   STN Patent Forums to be held July 19-22, 2004
 NEWS  8  Jun 28   Additional enzyme-catalyzed reactions added to CASREACT
 NEWS  9  Jun 28   ANTE, AQUALINE, BIOENG, CIVILENG, ENVIROENG, MECHENG,
                   and WATER from CSA now available on STN(R)
 NEWS 10  Jul 12   BEILSTEIN enhanced with new display and select options,
                   resulting in a closer connection to BABS

 NEWS EXPRESS  MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
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FILE 'HOME' ENTERED AT 17:00:36 ON 26 JUL 2004

=> file .biotech caplus
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                      ENTRY    SESSION
FULL ESTIMATED COST                                    0.21       0.21

FILES 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 17:00:53 ON 26 JUL 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

7 FILES IN THE FILE LIST

=> s kless H?/au
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L1          88 KLESS H?/AU

=> s l1 and primer extension
L2           1 L1 AND PRIMER EXTENSION

=> d all

L2   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   2001:168183  CAPLUS
DN   134:217979
ED   Entered STN:  09 Mar 2001
TI   Template-dependent nucleic acid polymerization using oligonucleotide
     triphosphate building blocks
IN   Kless, Hadar
PA   Yeda Research and Development Co. Ltd., Israel
SO   PCT Int. Appl., 69 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
IC   ICM C12Q001-68
CC   3-1 (Biochemical Genetics)
     Section cross-reference(s): 7
FAN.CNT 1
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   WO 2001016366     A2    20010308       WO 2000-IL515    20000829
     WO 2001016366     A3    20020117
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             CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
             HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
             LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
             SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
             YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
             DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
             CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     EP 1208236        A2    20020529       EP 2000-954880   20000829
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, LT, LV, FI, RO, MK, CY, AL
     JP 2003508063     T2    20030304       JP 2001-520911   20000829
     AU 773447         B2    20040527       AU 2000-67229    20000829
PRAI US 1999-387777    A     19990901
     WO 2000-IL515     W     20000829
AB   A novel use of a template-dependent polymerase in template-dependent
     5'→3' primer extension that uses
     oligonucleotide (typically dinucleotide) triphosphates as substrates in
     addition to, or instead of, nucleotide triphosphates is described.  The novel
     use is effected by employing the template-dependent polymerase for
     incorporating at least one oligonucleotide triphosphate onto a nascent
     oligonucleotide-3'-OH in a template-dependent manner.  If dinucleotides
     carrying modified bases are used as substrates, it becomes possible to
     incorporate 16 different modified nucleotides into a sequence, allowing
     the rapid generation of long oligonucleotides carrying an extensive array
     of altered bases and with novel properties in terms of information content
     and function, e.g. in the development of nanomachines.  Dinucleotides with
     3'-phosphorylation were found to be used as substrates by a number of DNA
     polymerases.  Incorporation was dependent upon base-pairing between both
     bases of the substrate and the template.
ST   primer extension polymerase phosphorylated
     dinucleotide substrate; DNA polymerase phosphorylated dinucleotide
     trinucleotide substrate primer extension;
     trinucleotide phosphorylated primer extension
     polymerase substrate
IT   Phosphorylation
```

(3'-, of dinucleotides for use as substrates in **primer extension**; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT Nucleic acid amplification (method)
(DNA, dinucleotides as substrate in; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT Nucleic acid bases
RL: RCT (Reactant); RACT (Reactant or reagent)
(analogs, incorporation into **primer extension** products; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT Mutation
(detection, **primer extension** with dinucleotides in; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT Oligonucleotides
RL: PNU (Preparation, unclassified); RCT (Reactant); PREP (Preparation); RACT (Reactant or reagent)
(dinucleotides, 3'-phosphorylated, as substrates for polymerases; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT Primers (nucleic acid)
RL: RCT (Reactant); RACT (Reactant or reagent)
(enzymic extension of; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT Nanomachines
(nucleic acid analogs containing base analogs for; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT DNA sequence analysis
(**primer extension** with dinucleotides in; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT Oligonucleotides
RL: PNU (Preparation, unclassified); RCT (Reactant); PREP (Preparation); RACT (Reactant or reagent)
(trinucleotides, 3'-phosphorylated, as substrates for polymerases; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT 9012-90-2, DNA-dependent DNA polymerase 9014-24-8, DNA-dependent RNA polymerase 9026-28-2, RNA-dependent RNA polymerase 9068-38-6, RNA-dependent DNA polymerase
RL: CAT (Catalyst use); USES (Uses)
(dinucleotides as substrate for; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)
IT 206344-38-9, 5: PN: WO0166691 SEQID: 5 unclaimed DNA 329385-22-0 329385-23-1 329385-24-2 329385-25-3 329385-26-4 329385-27-5 329385-28-6 329385-29-7 329385-30-0 329385-31-1 329385-32-2 329385-33-3
RL: PRP (Properties)
(unclaimed nucleotide sequence; template-dependent nucleic acid polymerization using oligonucleotide triphosphate building blocks)

=> s l1 and oligonucleotide
L3 2 L1 AND OLIGONUCLEOTIDE

=> d ibib abs l3

L3 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-08735 BIOTECHDS
TITLE: Use of a thermostable template-dependent polymerase for

incorporating **oligonucleotide** triphosphate onto nascent **oligonucleotide** 3'-end in template-dependent manner useful for applications in biotechnology and nanotechnology; DNA-polymerase, RNA-polymerase or reverse-transcriptase for use in DNA chip construction

AUTHOR: **Kless H**
PATENT ASSIGNEE: Yeda-Res.Develop.
LOCATION: Rehovot, Israel.
PATENT INFO: WO 2001016366 8 Mar 2001
APPLICATION INFO: WO 2000-IL515 29 Aug 2000
PRIORITY INFO: US 1999-387777 1 Sep 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-265894 [27]
AN 2001-08735 BIOTECHDS
AB Use of template-dependent polymerase (TDP) for incorporating at least one **oligonucleotide** triphosphate (OTP) onto a nascent **oligonucleotide**-3'-OH (NON) in a template-dependent way is claimed. Also claimed are: identifying TDP having increased activity among a library of mutated TDP, comprising screening the library using template-dependent polymerization of OTP for selecting mutant TDP exhibiting increased (M1) activity; assaying TDP for (M1) activity involving using template-dependent polymerization of OTP; exploiting the information transfer capacity of nucleic acid molecule comprising synthesizing a complementary nucleic acid molecule employing OTP instead of, or in addition to, nucleotide triphosphates as basic units for synthesis; exploiting the information transfer and functional capacities of nucleic acid molecules for DNA chip technology and nanotechnology, comprising contacting a nucleic acid template, TDP, NON, OTP and/or analog of OTP, where at least one component is immobilized on a DNA chip or nanodevice; a composition of 4N OPT; and a composition of OTP and NTP. The methods are used in pharmaceuticals, biocatalysis, and diagnosis. (69pp)

=> d l3 ibib abs l2
L2 IS NOT VALID HERE
For an explanation, enter "HELP DISPLAY".

=> d l3 ibib abs 2

L3 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:168183 CAPLUS
DOCUMENT NUMBER: 134:217979
TITLE: Template-dependent nucleic acid polymerization using **oligonucleotide** triphosphate building blocks
INVENTOR(S): **Kless, Hadar**
PATENT ASSIGNEE(S): Yeda Research and Development Co. Ltd., Israel
SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2001016366 | A2 | 20010308 | WO 2000-IL515 | 20000829 |
| WO 2001016366 | A3 | 20020117 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,

SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1208236 A2 20020529 EP 2000-954880 20000829
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL
JP 2003508063 T2 20030304 JP 2001-520911 20000829
AU 773447 B2 20040527 AU 2000-67229 20000829
PRIORITY APPLN. INFO.: US 1999-387777 A 19990901
WO 2000-IL515 W 20000829

AB A novel use of a template-dependent polymerase in template-dependent 5'→3' primer extension that uses **oligonucleotide** (typically dinucleotide) triphosphates as substrates in addition to, or instead of, nucleotide triphosphates is described. The novel use is effected by employing the template-dependent polymerase for incorporating at least one **oligonucleotide** triphosphate onto a nascent **oligonucleotide**-3'-OH in a template-dependent manner. If dinucleotides carrying modified bases are used as substrates, it becomes possible to incorporate 16 different modified nucleotides into a sequence, allowing the rapid generation of long oligonucleotides carrying an extensive array of altered bases and with novel properties in terms of information content and function, e.g. in the development of nanomachines. Dinucleotides with 3'-phosphorylation were found to be used as substrates by a number of DNA polymerases. Incorporation was dependent upon base-pairing between both bases of the substrate and the template.

=> d his

(FILE 'HOME' ENTERED AT 17:00:36 ON 26 JUL 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 17:00:53 ON 26 JUL 2004
L1 88 S KLESS H?/AU
L2 1 S L1 AND PRIMER EXTENSION
L3 2 S L1 AND OLIGONUCLEOTIDE

=> s oligonucleotide triphosphate
L4 5 OLIGONUCLEOTIDE TRIPHOSPHATE

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 4 DUP REM L4 (1 DUPLICATE REMOVED)

=> d ibib abs l5 1-4

L5 ANSWER 1 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
DUPLICATE 1
ACCESSION NUMBER: 2001-08735 BIOTECHDS
TITLE: Use of a thermostable template-dependent polymerase for incorporating **oligonucleotide triphosphate** onto nascent oligonucleotide 3'-end in template-dependent manner useful for applications in biotechnology and nanotechnology;
DNA-polymerase, RNA-polymerase or reverse-transcriptase for use in DNA chip construction
AUTHOR: Kless H
PATENT ASSIGNEE: Yeda-Res.Develop.
LOCATION: Rehovot, Israel.
PATENT INFO: WO 2001016366 8 Mar 2001
APPLICATION INFO: WO 2000-IL515 29 Aug 2000
PRIORITY INFO: US 1999-387777 1 Sep 1999

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-265894 [27]
AN 2001-08735 BIOTECHDS
AB Use of template-dependent polymerase (TDP) for incorporating at least one **oligonucleotide triphosphate** (OTP) onto a nascent oligonucleotide-3'-OH (NON) in a template-dependent way is claimed. Also claimed are: identifying TDP having increased activity among a library of mutated TDP, comprising screening the library using template-dependent polymerization of OTP for selecting mutant TDP exhibiting increased (M1) activity; assaying TDP for (M1) activity involving using template-dependent polymerization of OTP; exploiting the information transfer capacity of nucleic acid molecule comprising synthesizing a complementary nucleic acid molecule employing OTP instead of, or in addition to, nucleotide triphosphates as basic units for synthesis; exploiting the information transfer and functional capacities of nucleic acid molecules for DNA chip technology and nanotechnology, comprising contacting a nucleic acid template, TDP, NON, OTP and/or analog of OTP, where at least one component is immobilized on a DNA chip or nanodevice; a composition of 4N OPT; and a composition of OTP and NTP. The methods are used in pharmaceuticals, biocatalysis, and diagnosis. (69pp)

L5 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1979:435061 CAPLUS
DOCUMENT NUMBER: 91:35061
TITLE: Purification of fully protected oligonucleotide phosphotriester intermediates by gel filtration on Sephadex LH-60
AUTHOR(S): De Rooij, J. F. M., Jr.; Arentzen, R.; Den Hartog, J. A. J.; Van der Marel, G.; Van Boom, J. H.
CORPORATE SOURCE: Dep. Org. Chem., State Univ. Leiden, Leiden, 2300 RA, Neth.
SOURCE: Journal of Chromatography (1979), 171, 453-9
CODEN: JOCRAM; ISSN: 0021-9673
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Fully protected oligodeoxyribonucleotide phosphotriester intermediates were purified on an anal. or preparative scale with excellent recoveries by gel filtration chromatog. on Sephadex LH 60 with the eluent THF-MeOH (95:5). The purified compds. can be either completely deblocked to give oligonucleotides or selectively deblocked for use in coupling reactions in oligonucleotides synthesis. The Sephadex LH 60 was swollen in distilled THF for 2 h and packed into a 150 + 3 cm column. Samples were applied as 20-5% solns. in the eluent, and elution was by gravity flow, with relatively high flow rates (45-60 mL/h) still giving good resolution A typical run required .apprx.8 h. This method has many advantages over the commonly used procedure of short-column chromatog. on silica gel in CHCl3-MeOH.

L5 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1980:175136 CAPLUS
DOCUMENT NUMBER: 92:175136
TITLE: Mechanism of action of 2-5A in intact cells
AUTHOR(S): Hovanessian, Ara G.; Wood, John N.; Meurs, Eliane; Montagnier, Luc
CORPORATE SOURCE: Dep. Virol., Inst. Pasteur, Paris, Fr.
SOURCE: Regul. Macromol. Synth. Low Mol. Weight Mediators, [Proc. Workshop] (1979), 319-27. Editor(s): Koch, Gebhard; Richter, Dietmar. Academic: New York, N. Y.
CODEN: 42VHAO
DOCUMENT TYPE: Conference
LANGUAGE: English
AB A 2'-5' linked oligoadenylic acid triphosphate, 2-5A (10 nM), in which the trimer pppA 2'p5'A2p5'A [65954-93-0] is predominant, inhibited protein

formation in human, hamster, and mouse cells 50-85%. A secondary effect on the total RNA formation was observed several h after protein formation inhibition; however both of these effects were transient, and after an overnight recovery period recovered to levels of controls. Activation of a nuclease [9026-81-7] was detected after 2-5A treatment. Degradation of ribosomal and polyadenylated RNA was observed in exts. of 2-5A-treated cells. Exts. from 2-5A-pretreated cells had enhanced nuclease activity in vitro on incubation with exogenous RNA. Apparently, the mechanism of 2-5A in intact cells involves activation of a nuclease.

L5 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1977:121672 CAPLUS
DOCUMENT NUMBER: 86:121672
TITLE: Chemical and biological synthesis of the confronted nucleotide structure at the 5'-terminus of mRNA of CP virus
AUTHOR(S): Yamaguchi, Kazuo; Nakagawa, Iwao; Hata, Tsujiaki; Shimotohno, Kunitada; Shimotohno, Kumiko; Hiruta, Michiyo; Miura, Kinichiro
CORPORATE SOURCE: Dep. Life Chem., Tokyo Inst. Technol., Tokyo, Japan
SOURCE: Nucleic Acids Research, Special Publication (1976), 2(Symp. Nucleic Acids Chem., 4th, 1976), 151-4
CODEN: NARPD6; ISSN: 0309-1872
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The methylated blocked structure of the 5'-terminus of cytoplasmic polyhedrosis virus messenger RNA containing α,γ-dinucleoside triphosphate, m7G5'pppAm-G (m = Me), was prepared The biol. formation of the modified structure was studied with nucleotides including the non-methylated nucleotide structure.

=> d his

(FILE 'HOME' ENTERED AT 17:00:36 ON 26 JUL 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 17:00:53 ON 26 JUL 2004
L1 88 S KLESS H?/AU
L2 1 S L1 AND PRIMER EXTENSION
L3 2 S L1 AND OLIGONUCLEOTIDE
L4 5 S OLIGONUCLEOTIDE TRIPHOSPHATE
L5 4 DUP REM L4 (1 DUPLICATE REMOVED)

=> dinucleotide triphosphate
DINUCLEOTIDE IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s dinucleotide triphosphate
L6 15 DINUCLEOTIDE TRIPHOSPHATE

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7 12 DUP REM L6 (3 DUPLICATES REMOVED)

=> d ibib abs l7 1-12

L7 ANSWER 1 OF 12 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-13916 BIOTECHDS
TITLE: New expression system, useful as vaccine for treating cancer, comprises DNA molecule encoding interleukin-12 and costimulatory molecule, operably linked to promoter that

directs the expression of DNA molecule;
recombinant protein, vector expression in host cell, and polymerase chain reaction use in disease therapy, vaccine and gene therapy

AUTHOR: LEE S; KIM H
PATENT ASSIGNEE: LEE S; KIM H
PATENT INFO: US 2002018767 14 Feb 2002
APPLICATION INFO: US 2000-828825 27 Jul 2000
PRIORITY INFO: KR 2000-43498 27 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-239247 [29]
AN 2002-13916 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An expression system (I) comprising a DNA molecule encoding interleukin (IL)-12 and a costimulatory molecule, operably linked to a promoter, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a cancerous cell (II) transfected with (I); and (2) an anti-cancer vaccine (III) comprising (III) and a suitable excipient.

WIDER DISCLOSURE - Kits for eliciting immune response in a patient and vaccinating a patient against cancer, and their uses, are also disclosed.

BIOTECHNOLOGY - Preferred Compound: IL-12 is a single gene fusion of p35 and p40. The costimulatory molecule is B7-1, B7-2 or CD40L.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Vaccine; stimulator of T cells. Allogenic T lymphocytes (1 x 10 to the power of 6 cells/ml) were co-cultured with irradiated vector-transfected ME-180 (1,2,4, 8 x 10 to the power of 4 cells/ml) in 96 well flat-bottomed plates in the presence of 1 microgram/ml of PHA (undefined). In some cases, murine CD28-Fc was included to determine the direct effects of B7-1 expressed on vector-transfected ME-180. 48 hours later, each well received 1 micro Ci (3H)-thymidine, and after an additional 16 hour, cultures were harvested onto glass filters, and radioactivity was assessed by liquid scintillation. While a significant T cell costimulatory activity (50-100 fold higher counts per minute over background) was shown by cells transfected with pIRES-B7-1-IL12 series, only a minor (3 fold higher counts per minute over background) was detected on cells transfected with pIRES-IL-12-hB7-1 series. Furthermore, there was a dose-dependent inhibition of costimulatory activity by chimeric CD28-Fc protein, suggesting that major costimulation was provided directly by B7-1 on vector-transfected ME-180.

USE - (I) is useful for eliciting an anti-tumor immune response in a patient, by isolating cancerous cells from a patient, transecting the cancerous cells with (I), incubating the transfected cells under conditions such that IL-12 and the costimulatory molecules are expressed and eliciting an anti-tumor immune response in the patient by injecting the transfected cells into the patient after irradiating the cells to prevent replication. (I) is also useful for vaccinating an individual, by transfecting cancerous cells of a donor with (I), incubating the cells under conditions such that IL-12 and the costimulatory molecules are expressed, isolating native T cells from the individual and exposing them to transfected cancerous cells, to activate the T cells, separating the active T cells and injecting them into the patient (claimed). (I) is useful for in vitro generation of genetically modified human cancer cells for cancer therapy. These cells share phenotypes of both antigen presenting cells and cancer cells and are suitable as a cellular vaccine for certain types of cancer. (II) is useful for activating T cells for immunotherapeutic responses against primary or metastatic cancers.

ADMINISTRATION - (II) is administered through subcutaneous, intraperitoneal, intramuscular, intradermal or intravenous route. The dosage is 1 x 10 to the power of 4-1 x 10 to the power of 6 cells.

ADVANTAGE - Use of (I) in cancer therapy, does not require the

identification and purification of antigenic peptides, and it can be applied to almost every type of cancers. The cellular vaccine may not induce Graft versus Host Disease since cancer cells are derived from the self, and thus the method provides a new and safer therapeutic strategy for primary and metastatic cancer by activating patient's own immune system.

EXAMPLE - Both B7-1 and interleukin (IL)-12 genes were cloned by standard recombinant DNA techniques. The cDNA encoding the p35 and p40 chains of human IL-12 and human B7-1 were generated from 1 microgram/ml of lipopolysaccharide (LPS)-stimulated human peripheral blood monocytes or CD40 ligand and IL-4 stimulated human B cells by reverse transcriptase-polymerase chain reaction (RT-PCR). First, mRNA was isolated by oligo-dT MACS (undefined) column and subjected to first strand cDNA synthesis. Primers selected from the 5' and 3'-end of the coding sequences of each gene were designed to introduce restriction sites. PCR was done with PCR-premix containing PCR buffer, **dinucleotide triphosphate** (dNTP), TAQ polymerase and MgCl2. After cloning of B7.1, each gene was subcloned into PGEM vector generating pGEM-hp40, pGEM-hp35, and pGEM-hB7-1 and clones were verified by sequencing. Next, the PCR product of hB7-1 was excised at appropriate restriction sites and inserted into MCS A site on pIRES or into MCS B site excised with restriction enzymes generating pIRES (internal ribosome binding sites)-hB7-1 (A) and pIRES-hB7-1 (B). The cDNA for the single chain IL-12 fusion protein was constructed by linkage of the p40 and p35 cDNAs with synthetic linkers of 2,3 and 4 repeats of GGGGS containing NcoI restriction sites. The p40 from pGEM-hp40 was amplified with linker-containing primers producing p40L2, p40L3 and p40L4. The p35 was cloned downstream of the linker with restriction site. These products (about 1.6 kb) were cloned into pGEM-T easy vector generating pGEM-IL12.2, pGEM-IL12.3, and pGEM-IL12.4. PCR products from the three vectors were digested with the appropriate restriction enzymes and ligated into the MCS B on pIRES-hB7-1 (A) or into restriction site of MCS A on pIRES-hB7-1 (B) generating 2 series (pIRES-hB7-1-IL12 and pIRES-IL12-hB7-1 series) of 6 different expression vectors (pIRES-hB7-I-IL12.2, pIRES-hB7-1-IL12.3, and pIRES-hB7-1-IL12.4, pIRES-IL12.2-hB7-1, pIRES-IL12.3-hB7-1, and pIRES-IL12.4-hB7-1). Constructs were sequenced across all cloning junctions to determine the fidelity of recombination process. (76 pages)

L7 ANSWER 2 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:523291 BIOSIS
DOCUMENT NUMBER: PREV200200523291
TITLE: Method of promoting mucosal hydration with certain uridine, adenine and cytidine diphosphates and analogs thereof.
AUTHOR(S): Yerxa, Benjamin R. [Inventor]; Rideout, Janet L. [Inventor]; Jones, Arthur C. [Inventor, Reprint author]
CORPORATE SOURCE: Durham, NC, USA
ASSIGNEE: Inspire Pharmaceuticals, Inc.
PATENT INFORMATION: US 6436910 August 20, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 20, 2002) Vol. 1261, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Oct 2002
Last Updated on STN: 9 Oct 2002

AB A method and preparation for the stimulation of mucosal hydration in a subject in need of such treatment is disclosed. The method comprises administering to the mucosal surfaces of the subject a purinergic receptor agonist such as uridine 5'-diphosphate (UDP), dinucleotide triphosphates; cytidine 5'-diphosphate (CDP), adenosine 5'-diphosphate (ADP), or their therapeutically useful analogs and derivatives, in an amount effective to stimulate mucin secretion. Pharmaceutical formulations and methods of

making the same are also disclosed. Methods of administering the same would include: topical administration via a liquid, gel, cream, or as part of a contact lens or selective release membrane; or systemic administration via nasal drops or spray, inhalation by nebulizer or other device, oral form (liquid or pill), injectable, intra-operative instillation or suppository form. A method for facilitating the expectoration of sputum for the purpose of detecting cellular abnormalities indicative of lung disease is also disclosed.

L7 ANSWER 3 OF 12 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 1
ACCESSION NUMBER: 2001:833561 SCISEARCH
THE GENUINE ARTICLE: 481MW
TITLE: N-diphenylmethyl-2-propenamide: theoretical study of the structure and interaction with a DNA model system
AUTHOR: Barone G (Reprint); Saturnino C; De Martino G; Duca D; La Manna G
CORPORATE SOURCE: Univ Salerno, Dipartimento Sci Farmaceut, Via Ponte Don Melillo, I-84084 Salerno, Italy (Reprint); Univ Salerno, Dipartimento Sci Farmaceut, I-84084 Salerno, Italy; Univ Palermo, Dipartimento Chim Fis, I-90128 Palermo, Italy
COUNTRY OF AUTHOR: Italy
SOURCE: JOURNAL OF MOLECULAR STRUCTURE-THEOCHEM, (17 SEP 2001) Vol. 572, pp. 113-119.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0166-1280.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 11
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB N-diphenylmethyl-2-propenamide (NDP) was synthesised and characterised. Pharmacological in vitro tests pointed out that NDP had a cytotoxic activity on a human ovarian carcinoma comparable to that of doxorubicin. Hypothesising that this in vitro cytotoxic activity could be mainly due to intercalating interactions, between the drug and DNA fragments, ab initio calculations, at the Hartree-Fock (HF) level, were performed on the structure, and on the conformational properties of NDP, whereas its interaction with an (AC)(TG) **dinucleotide triphosphate** duplex (DD) was studied by the ONIOM method, at HF and PM3 level for NDP and DD, respectively. The supposed intercalation process with the DNA fragment was discussed in terms of the co-planarity of the aromatic rings present in the NDP molecule. (C) 2001 Elsevier Science B.V. All rights reserved.

L7 ANSWER 4 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:122495 BIOSIS
DOCUMENT NUMBER: PREV200100122495
TITLE: Enhancement of the anti-HIV activity of 3TC by depletion of dCTP.
AUTHOR(S): Roy, B. [Reprint author]; Clayette, P. [Reprint author]; Bosquet, N. [Reprint author]; Lemaire, G. [Reprint author]; Lepoivre, M. [Reprint author]
CORPORATE SOURCE: Oxydes d'azote, inflammation et immunite, UMR CNRS 8619, University Paris XI, bat 430, 91405, Orsay, France
SOURCE: Biochemical Society Transactions, (October, 2000) Vol. 28, No. 5, pp. A331. print.
Meeting Info.: 18th International Congress of Biochemistry and Molecular Biology. Birmingham, UK. July 16-20, 2000. International Union of Biochemistry and Molecular Biology; Federation of European Biochemical Societies; Biochemical Society.
CODEN: BCSTB5. ISSN: 0300-5127.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LANGUAGE: English
ENTRY DATE: Entered STN: 7 Mar 2001
Last Updated on STN: 15 Feb 2002

L7 ANSWER 5 OF 12 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 2
ACCESSION NUMBER: 1999:675733 SCISEARCH
THE GENUINE ARTICLE: 230VZ
TITLE: Semiempirical calculations on the interaction between dimethyltin(IV) and DNA model system
AUTHOR: Barone G; Ramusino M C; Barbieri R; LaManna G (Reprint)
CORPORATE SOURCE: UNIV PALERMO, DIPARTIMENTO CHIM FIS, GRP CHIM TEOR, VIA ARCHIRAFI 20, I-90123 PALERMO, ITALY (Reprint); UNIV PALERMO, DIPARTIMENTO CHIM FIS, GRP CHIM TEOR, I-90123 PALERMO, ITALY; IST SUPER SANITA, I-00161 ROME, ITALY; UNIV PALERMO, DIPARTIMENTO CHIM INORGAN, I-90123 PALERMO, ITALY
COUNTRY OF AUTHOR: ITALY
SOURCE: THEOCHEM-JOURNAL OF MOLECULAR STRUCTURE, (1 SEP 1999) Vol. 469, pp. 143-149.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0166-1280.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 20
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The interaction between a **dinucleotide triphosphate** duplex (DD), mimicking the DNA molecule, and the dimethyltin(IV) cation is studied by the semiempirical PM3 method. The results show that the interaction can occur involving the tin atom and the electron-donor centres of DD, requiring in some cases the presence of water molecules. In particular, the binding of the dimethyltin(IV) moiety with two adjacent phosphate oxygen atoms is allowed by the presence of water molecules coordinating to the tin atom. In this case the tin environment shows a geometry in agreement with Sn-119 Mossbauer and X-ray data. (C) 1999 Elsevier Science B.V. All rights reserved.

L7 ANSWER 6 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1990:425489 BIOSIS
DOCUMENT NUMBER: PREV199090086290; BA90:86290
TITLE: HIGH FIDELITY DNA SYNTHESIS BY THE THERMUS-AQUATICUS DNA POLYMERASE.
AUTHOR(S): ECKERT K A [Reprint author]; KUNKEL T A
CORPORATE SOURCE: LAB MOL GENETICS, NATL INST ENVIRONMENTAL HEALTH SCIENCES, PO BOX 12233, RESEARCH TRIANGLE PARK, NC 27709, USA
SOURCE: Nucleic Acids Research, (1990) Vol. 18, No. 13, pp. 3739-3744.
CODEN: NARHAD. ISSN: 0305-1048.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 22 Sep 1990
Last Updated on STN: 23 Sep 1990

AB We demonstrate that despite lacking a 3'→5' proofreading exonuclease, the Thermus aquaticus (Taq) DNA polymerase can catalyze highly accurate DNA synthesis in vitro. Under defined reaction conditions, the error rate per nucleotide polymerized at 70° C can be as low as 10-5 for base substitution errors and 10-6 for frameshift errors. The frequency of mutations produced during a single round of DNA synthesis of the lac Zα gene by Taq polymerase responds to changes in dNTP concentration, pH, and the concentration of MgCl2 relative to the total concentration of deoxynucleotide triphosphates present in the reaction. Both base substitution and frameshift error rates of <1/100,000

were observed at pH 5-6 (70° C) or when MgCl2 and deoxynucleotide triphosphates were present at equimolar concentrations. These high fidelity reaction conditions for DNA synthesis by the Taq polymerase may be useful for specialized uses of DNA amplified by the polymerase chain reaction.

L7 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:145611 CAPLUS
DOCUMENT NUMBER: 108:145611
TITLE: Occurrence of a novel nucleotide, Zpp5'A2'p, in rat liver extracts
AUTHOR(S): Buitrago, F.; Canales, J.; Sillero, M. A. Guenther; Sillero, A.
CORPORATE SOURCE: Inst. Invest. Biomed., Consejo Super. Invest. Cientificas, Badajoz, 06080, Spain
SOURCE: Biochemistry International (1988), 16(1), 175-84
CODEN: BIINDF; ISSN: 0158-5231
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The nucleotide Zpp5'A2'p was isolated from rat liver; Z is an unknown compound, probably a nucleoside. The preliminary structure of Zpp5'A2'p was elucidated by treatment with phosphodiesterase and/or alkaline phosphatase and anal. of the products of the reaction by HPLC. The following UV absorption spectral characteristics were determined at pH 7.0: for Zpp5'A2'p, γmax = 265 nm, A250/A260 = 0.76, A280/A260 = 0.83 and for Zp, γmax = 280 nm, A250/A260 = 0.88, A280/A260 = 1.46, where Ax is the absorbance at wavelength x. The molar extinction coefficient of Zp at 280 nm was 7.5 + 103 M-1 cm-1. The base of Zp could correspond to an indole derivative

L7 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1985:199751 CAPLUS
DOCUMENT NUMBER: 102:199751
TITLE: On the origin of sequence dependent structural changes in DNA
AUTHOR(S): Kothekar, V.; Kalia, A.; Yajnik, V.
CORPORATE SOURCE: Dep. Biophys., All-India Inst. Med. Sci., New Delhi, 110029, India
SOURCE: Progress in Clinical and Biological Research (1985), 172(Mol. Basis Cancer, Pt. B), 15-23
CODEN: PCBRD2; ISSN: 0361-7742
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The energetics of conformational hinges in DNA in response to environmental factors and base sequence were examined by calcn. of the energetics of oligonucleotides in different conformations and by conformational anal. Potential energy functions of hexanucleotide single- and double-stranded fragments with A, B, and Z conformations were determined The nucleotide sequence influenced both stacking and backbone interactions. Contributions of H bonding were also considered. Single-strand DNA had more pronounced energy variations than did double-stranded DNA; the A form exhibited greater variation than did the B and Z forms. Calcns. on a **dinucleotide triphosphate** model were performed to analyze the energetics of the backbone conformation of DNA. At a dielec. constant ε of 20, no sequence-specific effects were observed, but at ε = 4.0, sequence-specific conformational changes were seen; at ε = 2.0, helical structure was disrupted completely. Sequence specificity was primarily due to the electrostatic component of base phosphate interactions; ionic strength and binding of cations to the phosphate groups altered the backbone conformation. Finally, the influences of base orientation, sugar pucker, rotation around the C'3-C'4 bond, and the glycosyl torsional angle were addressed.

L7 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1977:536260 CAPLUS
DOCUMENT NUMBER: 87:136260
TITLE: Synthesis of oligonucleotide 5'-triphosphates
AUTHOR(S): Budker, V. G.; Zarytova, V. F.; Knorre, D. G.; Kobets, N. D.; Ryazankina, O. I.
CORPORATE SOURCE: Inst. Org. Chem., Novosibirsk, USSR
SOURCE: Bioorganicheskaya Khimiya (1977), 3(5), 618-25
CODEN: BIKHD7; ISSN: 0132-3423
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB PppUpUpU (ppp = tetrahydrogen triphosphate) was obtained by activation of the nucleotide monophosphate containing a protected 2'-OH group with N,N'-dicyclohexylcarbodiimide in DMF followed by treatment with Na4P2O7. Analogously obtained was the deoxyribonucleotide pppdTpdT.

L7 ANSWER 10 OF 12 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 75114785 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1090616
TITLE: The activity of oligonucleotides containing guanosine 5'-triphosphate in protein synthesis. I. The interaction of protein synthesis elongation factor I with cytidylyl (5'-3')-guanosine 5'-triphosphate.
AUTHOR: Allende J E; Allende C C
SOURCE: Journal of biological chemistry, (1975 Mar 25) 250 (6) 2056-61.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197506
ENTRY DATE: Entered STN: 19900310
Last Updated on STN: 19900310
Entered Medline: 19750606
AB The interaction of protein synthesis elongation factor 1 (EF-1) from wheat embryos and elongation factor Tu from Escherichia coli with cytidylyl(5'-3')guanosine 5'-triphosphate(pppGpC) has been studied. The dinucleotide 5'-triphosphate interacts strongly with EF-1 as evidenced by its capacity to inhibit the binding of [3H]GTP to the factor. The analogs pGpC and GpC do not interfere with GTP binding to EF-1 but guanosine 5'-triphosphate cyclic 2',3'-monophosphate and ppGpC are also potent inhibitors. The binding of the dinucleotide 5'-triphosphate to EF-1 was also demonstrated directly by the nitrocellulose retention method and by Sephadex G-50 fractionation using a radioactive analog iodinated with 125I in the 5 position of the cytosine of pppGpC. The **dinucleotide triphosphate** can replace GTP in the formation of a ternary complex EF-1-aminoacyl-tRNA-GTP and in its requirement for the binding of aminoacyl-tRNA to ribosomes catalyzed by EF-1. The absolute requirement for GTP in an in vitro polypeptide-synthesizing system can also be met by pppGpC and by guanosine 5'-triphosphate cyclic 5',3'-monophosphate. The bacterial factor EF-Tu differs drastically from eukaryotic EF-1 in its nucleotide specificity since EF-Tu only interacts slightly (if at all) with pppGpC. The low inhibition of [3H]GTP binding to EF-Tu by pppGpC could be due to a slight contamination in the latter compound.

L7 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1975:428502 CAPLUS
DOCUMENT NUMBER: 83:28502
TITLE: Synthesis of pppGpN type dinucleotide derivatives. 5' End sequence of some RNAs
AUTHOR(S): Simoncsits, A.; Tomasz, J.; Allende, J. E.
CORPORATE SOURCE: Inst. Biophys., Hung. Acad. Sci., Szeged, Hung.
SOURCE: Nucleic Acids Research (1975), 2(2), 257-63

CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English
AB PppGpN type (N = C, U, A) diribonucleotides were prepared in 50% yield by coupling guanosine 2',3'-cyclic phosphate 5'-triphosphate with the appropriate ribonucleoside in the presence of ribonuclease T1.

L7 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1970:400203 CAPLUS
DOCUMENT NUMBER: 73:203
TITLE: Structural analysis of nucleolar precursors of ribosomal ribonucleic acid. Studies on the 5'-terminal and alkali-resistant dinucleotides of nucleolar high molecular weight ribonucleic acid
AUTHOR(S): Choi, Yong Chun; Busch, Harris
CORPORATE SOURCE: Dep. of Pharmacol., Baylor Coll. of Med., Houston, TX, USA
SOURCE: Journal of Biological Chemistry (1970), 245(8), 1954-61
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To estimate the chain lengths based upon the 5' terminals and to provide information on methylated oligonucleotides, nucleolar 28, 35, and 45 S RNAs were subjected to alkaline hydrolysis and the resultant oligonucleotides were separated and identified. The 5' terminal of each RNA class was mainly (80%) the methylated **dinucleotide triphosphate** pCmpUp. In addition, smaller amts. of pCp (10%) and pAp (10%) were found. Based on the 5' terminal anal., the ratio of the approximate chain lengths of the polynucleotide chains is 1:1.5:2.5 in nucleolar 28, 35, and 45 S RNA, resp., and is in good agreement with ratios derived from hybridization studies and equilibrium sedimentation studies. An alkali-stable trinucleotide (UmpUmpCp) is present in all of the nucleolar RNA classes in amts. equimolar to the terminal pCmpUp. The 16 types of 2'-O-methylated alkali-stable dinucleotides present in the nucleolar 28, 35, and 45 S RNAs accounted for 1.2, 1.1, and 0.9% of the total residues in 28, 35, and 45 S nucleolar RNA, resp. From these values, and the cleavage patterns derived from the hybridization data, a working model of nucleolar 45 S RNA is presented in which the 28 S nucleolar RNA is placed at the 5' end of the mol.

=> d his

(FILE 'HOME' ENTERED AT 17:00:36 ON 26 JUL 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 17:00:53 ON 26 JUL 2004
L1 88 S KLESS H?/AU
L2 1 S L1 AND PRIMER EXTENSION
L3 2 S L1 AND OLIGONUCLEOTIDE
L4 5 S OLIGONUCLEOTIDE TRIPHOSPHATE
L5 4 DUP REM L4 (1 DUPLICATE REMOVED)
L6 15 S DINUCLEOTIDE TRIPHOSPHATE
L7 12 DUP REM L6 (3 DUPLICATES REMOVED)

=> template dependent polymerization
TEMPLATE IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s template dependent polymerization
L8 9 TEMPLATE DEPENDENT POLYMERIZATION

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9 6 DUP REM L8 (3 DUPLICATES REMOVED)

=> d ibib abs l9 1-6

L9 ANSWER 1 OF 6 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-10881 BIOTECHDS
TITLE: Producing detectably labeled nucleic acid, by enzymatically generating by **template dependent polymerization** oligonucleotide tagged nucleic acid and contacting tagged nucleic acid with labeled oligonucleotide;
tagged oligonucleotide, DNA array and DNA **template -dependent polymerization** for labeled DNA construction
AUTHOR: WOLBER P K; SHANNON K W
PATENT ASSIGNEE: WOLBER P K; SHANNON K W
PATENT INFO: US 2004005609 8 Jan 2004
APPLICATION INFO: US 2003-431335 6 May 2003
PRIORITY INFO: US 2003-431335 6 May 2003; US 2000-495152 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-167525 [16]
AN 2004-10881 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) detectably labeled nucleic acid, by enzymatically generating by **template dependent polymerization** an oligonucleotide tagged nucleic acid (I), where (I) comprises an oligonucleotide tag and contacting (I) with labeled oligonucleotide complementary to oligonucleotide tag under conditions sufficient for the labeled oligonucleotide to hybridize to oligonucleotide tag.

DETAILED DESCRIPTION - Producing (M1) a detectably labeled nucleic acid, involves enzymatically generating by **template dependent polymerization** an oligonucleotide tagged nucleic acid, where the oligonucleotide tagged nucleic acid comprises an oligonucleotide tag and contacting the oligonucleotide tagged nucleic acid with a labeled oligonucleotide complementary to the oligonucleotide tag under conditions sufficient for the labeled oligonucleotide to hybridize to the oligonucleotide tag, where a detectably labeled nucleic acid is produced. INDEPENDENT CLAIMS are included for the following: (1) producing (M2) a detectably labeled population of target nucleic acids from an initial nucleic acid sample, involves enzymatically generating a population of oligonucleotide tagged target nucleic acids from an initial nucleic acid sample, where the oligonucleotide tagged target nucleic acids in the population comprise the same oligonucleotide tag and contacting the population of oligonucleotide tagged target nucleic acids with labeled oligonucleotide complementary to the oligonucleotide complementary to the oligonucleotide tag of each oligonucleotide tagged target nucleic acid under hybridization conditions, where a detectable labeled population of target nucleic acids is produced; (2) detecting (M3) the presence of a nucleic acid analyte in a target sample, involves enzymatically generating an oligonucleotide tagged target nucleic acid from the nucleic acid analyte, where the oligonucleotide tagged target nucleic acid comprises an oligonucleotide tag and producing a hybridized complex that comprises the tagged target nucleic acid an oligonucleotide label and a probe nucleic acid, detecting the presence of the hybridized complex and relating the presence of the hybridized complex to the presence of the nucleic acid analyte in the sample, where the presence of the nucleic acid analyte in the sample is detected; (3) obtaining (M4) an expression profile for at least a representative number of genes in a cell, involves enzymatically generating a population of oligonucleotide

tagged nucleic acids from an mRNA sample derived from the cell, where each oligonucleotide tagged nucleic acids comprises an oligonucleotide tag, producing at least one hybridized complex comprising a tagged target nucleic acid, a labeled oligonucleotide and a probe nucleic acid stably associated with the surface of a solid support, detecting the presence of at least one hybridized complex on the array surface and deriving an expression profile for the cell from the detected hybridized complex, where the expression profile for at least a representative number of genes in the cell is obtained; (4) comparing (M5) the expression profiles of at least two distinct samples, involves enzymatically generating a first population of oligonucleotide tagged nucleic acids from an mRNA sample derived from a first sample, where each oligonucleotide tagged nucleic acid of the first population comprises a first oligonucleotide tag and a second population of oligonucleotide tagged nucleic acids from a mRNA sample derived from a second sample, where each oligonucleotide tagged nucleic acid of the second population comprises a second oligonucleotide tag that differs from a first oligonucleotide tag, hybridization the first and second population of oligonucleotide tagged target nucleic acids to an array of probe nucleic acids stably associated with the surface of a solid support, where the hybridizing occurs in the presence of first labeled oligonucleotide complementary to the first oligonucleotide tag of the first population and second labeled oligonucleotide complementary to the second oligonucleotide tag of the second population, where hybridized complexes comprising the oligonucleotide tagged target nucleic acids, probe nucleic acid and labeled oligonucleotides are produced on the surface of the array, detecting the presence of the hybridized complexes on the array surface, deriving an expression profile for each of the cells from the detected hybridized complexes and comparing the derived expression profiles of each of the cells, where the expression of the at least two distinct cells are compared; and (5) a kit (K1) for use in obtaining an expression profile for at least a representative number of genes in a cell comprising a first primer comprising an hybridization domain and an oligonucleotide tag and a first labeled oligonucleotide complementary to the oligonucleotide tag.

BIOTECHNOLOGY - Preferred Method: In (M1), the detectably labeled nucleic acid is directly detectable. The directly detectable label is fluorescent. In (M2), the nucleic acid sample is an mRNA sample. A primer that comprises an oligo dT domain or oligo dT region and the oligonucleotide tag is employed is the enzymatically generating step. The primer further comprises an RNA polymerase promoter domain. The population of detectably labeled target nucleic acids is labeled with a fluorescent label. In (M3), the probe is stably associated with the surface of a solid support. The probe is present on an array. The labeled oligonucleotide is fluorescently labeled. The primer further comprises an RNA polymerase promoter. (M3) further involves transmitting data obtained from at least one of the detecting and related steps to a remote location. In (M4), the first and labels are distinguishable and are fluorescent. Preferred Kit: In (K1), the hybridization domain is the oligo dT domain. The hybridization domain is a domain of random sequence. (K1) further comprises an array of probe nucleic acids stably associated with is surface of a solid support. (K1) further comprises a second primer comprising a hybridization domain and the second oligonucleotide tag having a sequence different from the first oligonucleotide tag and a second a labeled oligonucleotide, where the label of the second labeled oligonucleotide is distinguishable from the label of the first labeled oligonucleotide. (K1) further comprises an RNA polymers. The first labeled oligonucleotide is labeled with a fluorescent label. (K1) further comprises a computer readable storage medium on which is recorded on algorithm for designing oligonucleotide tag sequences.

USE - (M1) is useful for producing a detectably labeled nucleic acid (claimed). (M1) is useful for hybridizing two or more distinct target nucleic acid population e.g., in comparative gene expression analysis in mammalian cells or cell populations such as blood or tissue derived from

brain, spleen, bone, heart etc.

EXAMPLE - Tag sequence were introduced as a linker in the primer promoters. The rarest codons in any organism were the stop codons TAA (ochre), TAG (amber) and TGA (opal). An initial list of 3127 unique 27mer concatamers yielded 16 candidates. The 16 candidates were than screened for subsets that possessed minimal potential for cross-hybridization. As a result, 2 T7(tag)T18VN primers were synthesized and were employed to generates oligonucleotide tagged target nucleic acid from an initial yeast mRNA sample. Oligonucleotide tagged target nucleic acid was generated for two different yeast poly A+RNA samples. For sample 1, T7 (tag1) T18VN was employed as the primer-tag-promoter, for sample to T7 (tag2) T18VN was employed as the primer-tag-promoter. Labeled oligonucleotides were synthesized. An array displaying probes for a representative number of yeast genes was contacted with both populations of tagged target nucleic acids and a molar excess of Cy3-ctag1 and Cy5-ctag2, and the targets were allowed to hybridized to the probe nucleic acids in the presence of the labeled oligonucleotides. Result showed that gene expressed in yeast cell 1 were identified by detecting complexes labeled with Cy3 and genes expressed in yeast cell 2 were identified by detecting complexes labeled with Cy5. (14 pages)

L9 ANSWER 2 OF 6 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-25262 BIOTECHDS
TITLE: Determining presence of specific nucleic acid variant, useful e.g. for diagnosis, by allele-specific amplification with a discriminating primer having a nucleotide modified at position 4 of the sugar;
DNA primer and polymerase chain reaction for sequence polymorphism detection and disease monitoring or prognosis
AUTHOR: PINGOUD A; HAHN M; TEWS B; WILHELM J; FRIEDHOFF P; MARX A; STRERATH M; SUMMERER D
PATENT ASSIGNEE: ROCHE DIAGNOSTICS GMBH; HOFFMANN LA ROCHE and CO AG F
PATENT INFO: WO 2003072814 4 Sep 2003
APPLICATION INFO: WO 2003-EP1725 20 Feb 2003
PRIORITY INFO: EP 2002-4221 26 Feb 2002; EP 2002-4221 26 Feb 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-721786 [68]
AN 2003-25262 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Determining presence of at least one sequence variant (SV) in one or more target nucleic acids (NA) in a sample, is new.

DETAILED DESCRIPTION - Method for determining presence of at least one sequence variant (SV) in one or more target nucleic acids (NA) in a sample comprises: (a) combining deoxynucleotide triphosphates (dNTP), an agent (I) for **template-dependent polymerization** of dNTP (e.g. a DNA polymerase); at least one discriminating primer (DP) containing at last one discriminating nucleotide (nt) residue, using one DP for each variant, and at least one additional primer (AP); (b) performing primer extension so that extension products are formed from DP only if the corresponding variant is present; (c) separating EP; (d) cyclic repetition of (b) and (c) to produce an amplification product (Amp), e.g. by PCR; and (e) detecting SV from presence of Amp. At least one residue of at least one DP is substituted at 4'-position of (deoxy)ribose; DP are complementary to specific SV; AP is complementary to a primer extension product of DP; and the variant being detected is complementary to at least 3'-terminal, proxi-terminal or proxi-proxi-terminal nt of DP. An INDEPENDENT CLAIM is also included for a kit for the process, containing at least one each of DP and AP.

BIOTECHNOLOGY - Preferred Process: Primer extension and amplification are particularly real-time (reverse transcription) PCR, with reaction monitored using fluorescently labeled probes. Alternatively, detection uses a DNA-binding dye. Preferred Kits: These may also contain dNTP and DNA polymerase.

USE - The method is used in molecular biology, diagnosis and prognosis to detect specific alleles, particularly point mutations or polymorphisms.

ADVANTAGE - The method improves specificity of detection of a particular variant (in known methods some primer extension can occur even from a mismatched primer, leading to false positive results), and the discriminating nucleotide need not be 3'-terminal.

EXAMPLE - A reaction mixture comprised the 36-mer template (36; X = A or G) and the 32phosphorus-labeled primer (25) in which the 3'-terminal T was 4'-methyl modified. It was subjected to conventional primer extension with Taq polymerase and the reaction products detected by gel electrophoresis and phosphor-imaging. Primer extension occurred only when X was A, but when the primer was replaced by a similar sequence with unmodified 3'-terminal T, both sequences (36) were amplified. 5'-GTGGTGCGAAATTTCTGACAGACAT (25) 5'-CACCACGCTTTAAAGACTGTCTGTXCTGTCGTCTGC GTG (36). (40 pages)

L9 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-07641 BIOTECHDS
TITLE: Producing detectably labeled nucleic acids for use in gene expression analyses, comprises enzymatically generating oligonucleotide tagged nucleic acids and contacting the nucleic acid with a labeled oligonucleotide;
labeled DNA probe preparation for DNA array construction and gene expression profiling
AUTHOR: WOLBER P K; SHANNON K W
PATENT ASSIGNEE: WOLBER P K; SHANNON K W
PATENT INFO: US 2002142313 3 Oct 2002
APPLICATION INFO: US 2001-861035 18 May 2001
PRIORITY INFO: US 2001-861035 18 May 2001; US 2000-495152 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-174134 [17]
AN 2003-07641 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Producing detectably labeled nucleic acids, comprises enzymatically generating (by **template dependent polymerization**) an oligonucleotide tagged nucleic acid comprising an oligonucleotide tag (OT), and contacting the nucleic acid with a labeled oligonucleotide complementary to the OT under conditions sufficient for the labeled oligonucleotide to hybridize to the OT, thus, producing a detectably labeled nucleic acid.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) a method of producing a detectably labeled population of target nucleic acids from an initial nucleic acid sample; (2) a method of detecting the presence of a nucleic acid analyte in a target sample; (3) a method for obtaining an expression profile for at least a representative number of genes in a cell; (4) a method for comparing the expression profiles of at least 2 distinct samples; and (5) a kit for use in obtaining an expression profile for at least a representative number of genes in a cell, comprising a first primer having a hybridization domain and an OT, and a first labeled oligonucleotide complementary to the OT.

BIOTECHNOLOGY - Preferred Method: In producing detectably labeled nucleic acids, the nucleic acid is directly detectable. The directly detectable label is fluorescent. Producing a detectably labeled population of target nucleic acids from an initial nucleic acid sample, comprises enzymatically generating a population of oligonucleotide tagged target nucleic acids from an initial nucleic acid sample, where the oligonucleotide tagged target nucleic acids in the population comprise the same oligonucleotide tag; and contacting the population of oligonucleotide tagged target nucleic acids with labeled oligonucleotides complementary to the oligonucleotide tag of each oligonucleotide tagged target nucleic acid under hybridization conditions, thus, producing a detectably labeled population of target nucleic acids. The nucleic acid

sample is an mRNA sample. A primer that comprises an oligo dT domain and the oligonucleotide tag is employed in the enzymatically generating step. The primer further comprises an RNA polymerase promoter domain. The detectably labeled population of target nucleic acids is labeled with a fluorescent label. Detecting the presence of a nucleic acid analyte in a target sample, comprises enzymatically generating an oligonucleotide tagged target nucleic acid from the nucleic acid analyte, where the oligonucleotide tagged target nucleic acid comprises an oligonucleotide tag; producing a hybridized complex that comprises the tagged target nucleic acid, an oligonucleotide label, and a probe nucleic acid; detecting the presence of the hybridized complex; and relating the presence of the complex to the presence of the nucleic acid analyte in the sample, thus the presence of the nucleic acid analyte in the sample is detected. The probe is present on an array, and is stably associated with the surface of a solid support. The method further comprises transmitting the data obtained from at least one of the detecting and related steps to a remote location. Obtaining an expression profile for at least a representative number of genes in a cell, comprises enzymatically generating a population of the oligonucleotide tagged nucleic acids from an mRNA sample derived from the cell; producing at least one hybridized complex cited above; detecting the presence of the hybridized complex on the array surface; and deriving an expression profile for the cell from the detected hybridized complex, thus, obtaining the expression profile for a representative number of genes. The method further comprises transmitting the data obtained from at least one of the detecting and deriving steps to a remote location. Comparing the expression profiles of at least 2 distinct samples, comprises enzymatically generating a first and a second population of oligonucleotide tagged nucleic acids from an mRNA sample derived from a first and a second sample, respectively, where each oligonucleotide tagged nucleic acid of the first population comprises a first oligonucleotide tag, and where each oligonucleotide tagged nucleic acid of the second population comprises a second oligonucleotide tag that differs from the first oligonucleotide tag; hybridizing the first and second populations of oligonucleotide tagged target nucleic acids to the array of probe nucleic acids cited above, where the hybridizing occurs in the presence of the first oligonucleotide complementary to the first oligonucleotide tag of the first population, and the second labeled oligonucleotide complementary to the second oligonucleotide tag of the second population, where the hybridized complexes cited above are produced on the surface of t he array; detecting the presence of the complexes on the array surface; deriving an expression profile for the cells from the detected hybridized complexes; and comparing the derived expression profiles of each of the cells. The first and the second labels are distinguishable. The first and second labels are fluorescent. Preferred Kit: The kit further comprises an array of probe nucleic acids cited above, an RNA polymerase, and a computer readable storage medium where an algorithm for designing oligonucleotide tag sequences is recorded. It also comprises a second primer having a hybridization domain and a second oligonucleotide tag that has a sequence different from the first oligonucleotide tag, and a second labeled oligonucleotide distinguishable from the first labeled oligonucleotide. The primer further comprises an RNA polymerase promoter. The hybridization domain is an oligo dT domain or a domain of random sequence.

USE - The method is useful for indirect labeling of image or target nucleic acids for use in gene expression analyses, in detecting the presence of a nucleic acid analyte in a target sample, in obtaining an expression profile for a representative number of genes in a cell, and in comparing the expression profiles of 2 distinct samples. The kit is useful in carrying out the above method.

EXAMPLE - No example given.(14 pages)

L9 ANSWER 4 OF 6 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-08735 BIOTECHDS

TITLE: Use of a thermostable template-dependent polymerase for incorporating oligonucleotide triphosphate onto nascent oligonucleotide 3'-end in template-dependent manner useful for applications in biotechnology and nanotechnology; DNA-polymerase, RNA-polymerase or reverse-transcriptase for use in DNA chip construction
AUTHOR: Kless H
PATENT ASSIGNEE: Yeda-Res.Develop.
LOCATION: Rehovot, Israel.
PATENT INFO: WO 2001016366 8 Mar 2001
APPLICATION INFO: WO 2000-IL515 29 Aug 2000
PRIORITY INFO: US 1999-387777 1 Sep 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-265894 [27]
AN 2001-08735 BIOTECHDS
AB Use of template-dependent polymerase (TDP) for incorporating at least one oligonucleotide triphosphate (OTP) onto a nascent oligonucleotide-3'-OH (NON) in a template-dependent way is claimed. Also claimed are: identifying TDP having increased activity among a library of mutated TDP, comprising screening the library using **template-dependent polymerization** of OTP for selecting mutant TDP exhibiting increased (M1) activity; assaying TDP for (M1) activity involving using **template-dependent polymerization** of OTP; exploiting the information transfer capacity of nucleic acid molecule comprising synthesizing a complementary nucleic acid molecule employing OTP instead of, or in addition to, nucleotide triphosphates as basic units for synthesis; exploiting the information transfer and functional capacities of nucleic acid molecules for DNA chip technology and nanotechnology, comprising contacting a nucleic acid template, TDP, NON, OTP and/or analog of OTP, where at least one component is immobilized on a DNA chip or nanodevice; a composition of 4N OPT; and a composition of OTP and NTP. The methods are used in pharmaceuticals, biocatalysis, and diagnosis. (69pp)

L9 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:895523 CAPLUS
DOCUMENT NUMBER: 124:56750
TITLE: **Template-dependent polymerization** of amino acids by utilizing protein synthesis systems
AUTHOR(S): Nitta, Itaru; Ueda, Takuya; Watanabe, Kimitsuna
CORPORATE SOURCE: Grad. Sch., Tokyo Univ., Tokyo, Japan
SOURCE: Kobunshi (1995), 44(9), 604-7
CODEN: KOBUA3; ISSN: 0454-1138
PUBLISHER: Kobunshi Gakkai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review with 23 refs. discussed the current status of the title polymerization in terms of applications and fundamentals.

L9 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 79201096 MEDLINE
DOCUMENT NUMBER: PubMed ID: 450717
TITLE: The effect of antibiotics on the T4 polynucleotide ligase catalyzed **template dependent polymerization** of oligodeoxythymidylates.
AUTHOR: Kalisch B W; van de Sande J H
SOURCE: Nucleic acids research, (1979) 6 (5) 1881-94.
Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 197908
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19980206
Entered Medline: 19790816

AB The poly(dA) dependent T4 polynucleotide ligase catalyzed polymerization of oligodeoxythymidylates is dependent upon duplex stability. The antibiotics ethidium bromide, netropsin and Hoechst 33258 stabilize the duplex poly(dA) . P(dT)n (n = 6-10) to thermal denaturation. Ethidium bromide to DNA ratio of 1.25 and netropsin or Hoechst 33258 to DNA ratio of 0.1 the Tm of d(pT) 10 . poly (dA) was increased by 10 degrees and 25 degrees C respectively. The T4 polynucleotide ligase activity was not inhibited under these conditions and temperature optimum of joining of d(pT) 10 . poly(dA) was increased 5 degrees to 10 degrees by the binding of the antibiotics. Duplexes containing shorter oligodeoxythymidylates required lower concentrations of the antibiotics netropsin or Hoechst 33258 to show no inhibition of T4 polynucleotide ligase. The temperature optima of joining the duplexes d(pT)6 . POLY(DA) and d(pT) 8 . poly(dA) were increased by 5 degrees C upon binding of the antibiotics. Polyacrylamide gel analysis of the T4 polynucleotide ligase catalyzed joining of the oligodeoxythymidylates showed that the presence of antibiotics affected the product distribution of the polymerized oligomers.